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Inducing the migration behavior of endothelial cells by tuning the ligand density on a density-gradient poly(ethylene glycol) surface

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ABSTRACT

The migration of endothelial cells (ECs) is crucially important for many biological processes, including early embryonic vasculogenesis, wound healing and angiogenesis. To investigate the effect of the surface poly(ethylene glycol) (mPEG-CHO) density on the migration of ECs, we developed a convenient and effective method to fabricate a series of silicon slides with graded PEG densities on their surfaces based on gradual treatment with 3-glycidoxypropyltrimethoxysilane (GPTMS), backfilling with 3-aminopropyltriethoxysilane (APTES) and subsequent conjugation of m-PEG. The PEG gradient was confirmed by X-ray photoelectron spectrometry (XPS), contact angle measurement and spectroscopic ellipsometry and determined to range from 0.56 to 0.75 chains/nm^2. The impact of the PEG gradient on the EC migration was evaluated by real-time observation *via* a time-lapse phase-contrast microscope. ECs adhered to the silicon surfaces with high and modest PEG densities displayed a higher tendency of migration than those on corresponding non-graded samples. The results suggest that the motility of ECs could be modulated by the PEG gradient. This study would be helpful for understanding cell-substrate interactions.

1. Introduction

In the past decades, significant efforts have been focused on investigating the migration of endothelial cells (ECs), as it is not only essential for embryonic development [1], wound repair [2] and tumorigenesis [3], but it is also crucial for angiogenesis in tissue repair [4]. Angiogenesis is the essential process of the formation of blood vessels to provide the necessary nutrients and oxygen to the implanted biomaterials and/or tissue-engineered structures for their enduring performance and increase the success rate of implantation [5].

Cell migration is a complicated process that is largely determined by the substrate, but it could also be affected by various stimuli, including biochemical and biophysical signals [6,7]. Many previous studies demonstrated that biophysical cues (wettability, stiffness, pore size) could modulate cell migration [8–12]. For instance, Vincent et al. found that mesenchymal stem cells (MSCs) migrated to the stiffest region on polyacrylamide-functionalized substrates [13]. Faia-Torres et al. confirmed that polycaprolactone (PCL) roughness strongly enhanced the osteogenic commitment and accelerated the differentiation of hBM-MSCs when they investigated the cell behavior of human bone-marrow MSCs grown onto PCL substrates with varied roughnesses [14]. In addition, a great number of surface-immobilized proteins and/or growth factors such as laminin [15], fibronectin (FN) [16], arginine-glycine-aspartic acid (RGD) [17] and vascular endothelial growth factor (VEGF) [18] could act as biochemical cues and accurately regulate cell migration. Previously, we manufactured collagen-functionalized poly(D, L-lactic acid) films with varied densities that have a strong impact on EC migration [19]. However, biological molecules are usually very expensive and susceptible to denaturation, which impedes their wide application [20].

It has been reported that biomaterials with biochemical or biophysical cues could induce cell migration *in vitro* [21]. Many studies also showed that biochemical cues could affect cell behavior by attaching proteins from their cellular environment [22,23]. Poly(ethylene glycol) (PEG) has proven to be a biocompatible material and has been extensively used in biomedical applications. The PEG chains are resistant to protein adsorption due to their superb hydrophilicity [24]. However, few studies emphasized the effect of PEG functionalization on EC migration.

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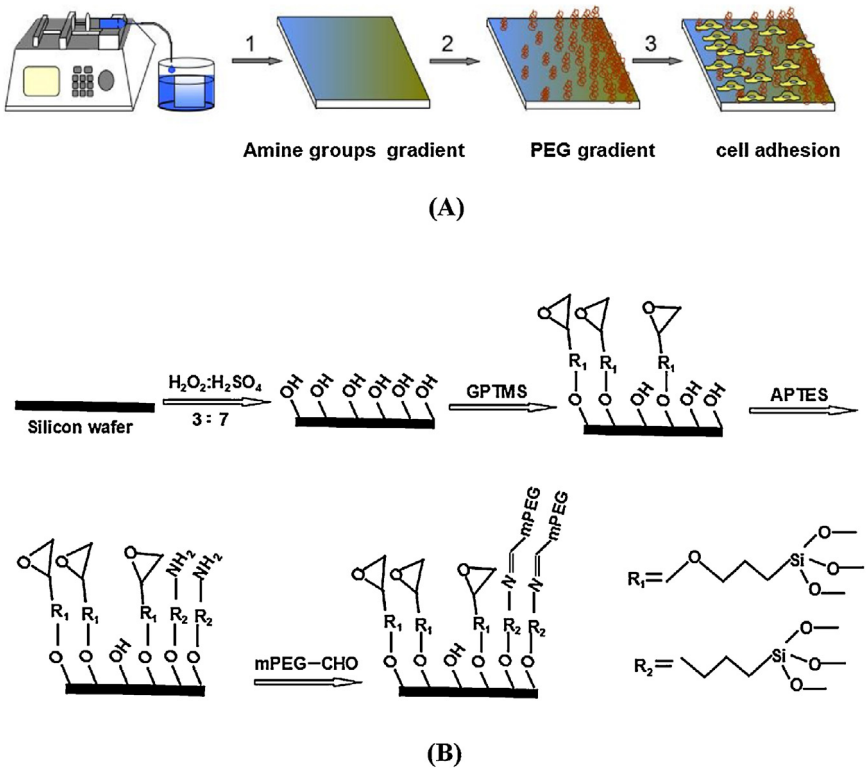

Fig. 1. (A) Schematic illustration showing the experimental concept; and (B) chemical reaction routes for the immobilization of poly(ethylene glycol) (PEG).

In this work, we fabricated a silicon surface with a graded PEG density using an efficient method. With the help of a syringe pump, silicon slides with graded amino density were first prepared, and mPEG-CHO was then grafted onto the amino-functionalized surfaces. The alignment and migration of endothelial cells in response to the PEG deposition were further observed *in vitro*.

2. Materials and methods

2.1. Materials

Methoxy PEG propionaldehyde (mPEG-CHO, Mw 2 kD) was purchased from Meilun Biotech Ltd., Co. (Dalian, China). 3-Aminopropyltriethoxysilane (APTES) and 3-glycidoxypropyltrimethoxysilane (GPTMS) were purchased from J&K Company (Beijing, China). Rhodamine-phalloidin and Hoechst 33258 were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Other chemicals were provided by the Oriental Chemicals Co., Ltd. (Chongqing, China). All chemicals were of analytical grade and used without further treatment if not otherwise mentioned. The water used in this study was of high purity (>18.2 MΩ cm, Millipore Milli-Q system).

2.2. Silane treatment

The GPTMS and APTES were dissolved in dry toluene to obtain 0.25% (v/v) and 1% (v/v) solutions, respectively. The silicon slides were cut into 10 mm × 10 mm pieces and were washed with toluene, acetone, alcohol and water under ultrasonication treatment in said order. Then, the samples were immersed into "piranha" solution (a mixture of 30% of hydrogen peroxide and 70% sulfuric acid (v/v). **Caution: the piranha solution is strongly corrosive and should be handled carefully!**) [21]. Finally, the samples were cleaned with distilled water 6 times and dried under a nitrogen flow immediately.

To obtain silicon slides with amino end groups, the cleaned materials were sequentially immersed into the GPTMS and APTES solutions for set periods of time. To produce samples with even amino distributions, the silicon slides were first treated with 0.25% GPTMS solution at 80 °C for 0, 10, 20, 40, or 80 min. After washing with toluene 6 times, the substrates were steeped in 1% APTES solution at 80 °C for 3 h. After that, the samples were sequentially cleaned in toluene, ethanol and water 6 times each. The treated silicon substrates were dried in an oven at 60 °C for 4 h. To produce silicon substrates with an amino gradient, the cleaned materials were put into a vertical glass vessel with a flat bottom to keep them upright at all times. The GPTMS gradient on the 10 mm silicon film was formed by injecting GPTMS solution into the vessel with a syringe pump at a constant rate of 50 μL/min. The inner diameters of the vessel and the injection syringe were 25 mm and 15 mm, respectively. A 10-mm slide with a GPTMS gradient could be obtained in 80 min (Fig. 1A). Next, the samples were immersed in APTES solution for back-filling at 80 °C for 3 h.

2.3. mPEG grafting

mPEG-CHO (2 mg/mL) was dissolved in phosphate buffer solution (PBS, pH 7.4) that contained 0.6 M K_2SO_4 [25]. Then, the silicon substrates with amino end groups were immersed into the mPEG-CHO solution at 60 °C for 12 h. After that, the samples were cleaned with Milli-Q water for 4 h under shaking to remove the physically adsorbed mPEG molecules. The silicon substrates treated with GPTMS, APTES and mPEG following the above steps were denoted as GPTMS-Si, APTES-Si, and PEG-Si, respectively.

2.4. Contact angle measurement

The static water contact angles of different samples were measured at room temperature with a Model 200 video-based optical system (Future Scientific Co. Ltd., Taiwan, China) using the sessile